

NORTHROP EXHIBIT B

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CLASS CODE

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UNIVERSITY OF CALIFORNIA, BERKELEY
OFFICE OF TECHNOLOGY LICENSING
INVENTION AND TECHNOLOGY DISCLOSURE

(see instructions on reverse side)

CASE NUMBER

B192-011

LOG DATE

COMPLETE ITEMS 1-4 - USE ADDITIONAL SHEETS AS NECESSARY

1. TITLE OF INVENTION

MICROINSTRUMENTATION - BASED POLYMERASE CHAIN
REACTION (PCR) DIAGNOSTICS

2. INVENTOR(S)

TITLE

CAMPUS UNIT OR MAILING ADDRESS

M. ALLEN NORTHROP

POST DOCTORAL
FELLOW (LLNL);
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(UCB)

LLNL; BSAC

BSAC; EECS DEPT.

RICHARD M. WHITE

PROFESSOR

3. CONTRACT OR GRANT NO(S)

SPONSOR(S)

PRINCIPAL INVESTIGATOR

N/A

4. EVENTS

A. Initial idea

DATE

REFERENCES & COMMENTS

B. First description of complete invention, oral or written
(conception)*C. First successful demonstration, if any (first actual reduc-
tion to practice)*D. First publication containing full description of invention
(establishment of publication bar)*

E. External oral disclosures

TELEPHONE CONVER-
SATION BETWEEN THE
INVENTORS
THIS DISCLOSURE
ATTACHED

NOT YET

NOT YET

NOT YET

5. BRIEF ABSTRACT OF INVENTION - ATTACH DETAILED DESCRIPTION

INVENTION CONCERNS APPLICATION OF MICRO-
STRUCTURES TO INSTRUMENT FOR AMPLIFICATION OF
DNA, ETC. A SPECIFIC EMBODIMENT EMPLOYING THE
LAMB-WAVE ULTRASONIC TECHNOLOGY IS SHOWN.

KEYWORDS (OTL USE ONLY)

6. INVENTION SUBMITTED BY:

MAN

Inventor's Signature

Date

CI

RAIW

Co-Inventor's Signature

Date

Co-Inventor's Signature

Date

Co-Inventor's Signature

Date

INVENTION DISCLOSED AND UNDERSTOOD BY:

Witness' Signature

Date

Print Name

Reviewed by:

Licensing Associate

Date

See instructions on back.

Please have PI sign if PI is not an inventor.

UCB OTL

AUG - 6 1991

OTL #1 1/1/90

Invention Disclosure Statement

Title: Microinstrumentation-based Polymerase Chain Reaction (PCR) Diagnostics

Inventors:

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Richard M. White
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Date:

Background:

The polymerase chain reaction (PCR) is a method by which a single molecule of DNA (or RNA) of an organism can be selectively amplified several millionfold within a few hours. This well-established procedure is based on the repetition of heating (denaturing) and cooling (annealing) cycles in the presence of the original DNA molecule, specific DNA primers, dNTPS, and DNA polymerase enzymes. Each cycle produces a doubling of the target DNA segment, leading to an exponential accumulation of the target segment. The generalized procedure involves: 1) processing of the sample to release target DNA molecules into a crude extract, 2) addition of an aqueous solution containing enzymes, buffers, deoxyribonucleotide triphosphates (dNTPS), and two oligonucleotide primers, 3) thermal cycling of the reaction mixture at two or three temperatures (i.e., 94, 72, and 37-54 °C) for typically 20 to 30 cycles, and 4) amplified DNA detection. Intermediate steps are introduced in some assays to incorporate signal-producing and/or surface-binding primers, and to purify the reaction products (e.g., electrophoresis or chromatography). Reaction volumes and times are typically on the order of tens of μ Ls and one to two hours, respectively. PCR-based technology has been applied to a variety of analyses, including environmental and industrial contaminant identification, medical diagnostics, and biological research.

Monolithic microfabrication technology has advanced to the point where a variety of micro-scale components can be made that have electrical, mechanical, optical, chemical, and thermal capabilities. For example, devices have been fabricated that can pump, heat, cool, and mix microliter quantities of solids and liquids. As well, micro-scale optical and electromechanical/chemical physical and chemical sensors have been developed such as fiber optic probes and Lamb-wave sensors. The integration of these devices into systems allows the development of analytical instruments on a micro-scale. The advantages of such integrated devices include the ability to manufacture them in batch quantities with high precision, yet low cost. Their inherent small size also provides significant advantage in that they would be able to perform highly automated *in situ* analyses.

Invention Concept

The invention disclosure herein concerns the application of microinstrumentation to PCR. The small analytical and reaction volumes of PCR make it an ideal diagnostic technique for

implementation on micro-devices. Such a system could contain reservoirs of reagents, agitation and mixing devices to process the target cells, pumps to carry solid and/or fluid reagents to mixing chambers, heaters and coolers to perform the denaturing and annealing cycles, optical and/or electromechanical/chemical sensors to discriminate the reagents and products of the reaction, and separation devices to purify reactants and products. Feedback control via integrated sensors could also be incorporated directly into the system.

Many or all of these devices could be made from microfabrication technology and could process micro- to picoliter volumes. By the selection and integration of appropriate microfabricated devices, a precise and reliable reaction and analysis instrument for PCR-based diagnostics could be devised. A schematic diagram of an example of one such possible system is presented in Figure 1. Several to many of these micro-instruments could be manufactured on a wafer and could run in parallel, allowing the processing and analysis of several target agents and controls. Target DNA detection methodology could include either an optical, electromechanical, electrochemical, or a combination sensing device. Detection signals could be processed and stored with microelectronic devices.

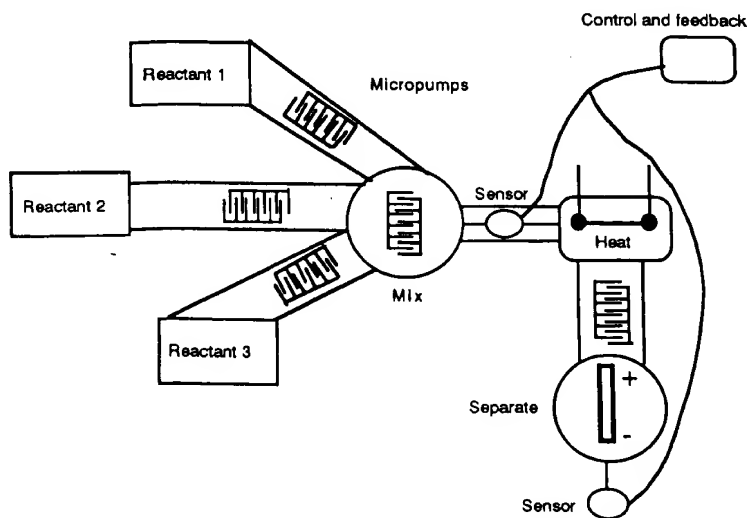
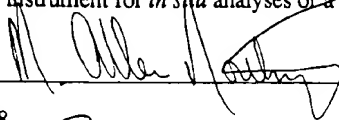


Figure 1. An example of an integrated microinstrument.

In summary, in this disclosure we describe an integrated microsystem and analytical instrument to perform PCR-based diagnostic methodology. The amplification process from minute sample sizes and reaction volumes, and specific reaction sequence of the PCR technique plays favorably into the micro-device capabilities of on-going microfabrication technology. The development of this integrated micro-PCR system will lead to a highly automated, miniaturized, analytical instrument for *in situ* analyses of a variety of samples.

Inventors:

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Witnesses:



Date _____



Date _____

SUMMARY OF LAMB-WAVE DEVICE TECHNOLOGY

R. M. White --

Device Applications: The basic Lamb-wave device fabricated by planar processing (low-stress LPCVD silicon nitride, RF planar-magnetron sputtered ZnO, aluminum) may be used in three major ways announced to date:

- 1 Physical sensing: Velocity affected (differential pressure, temperature); attenuation affected (fluid viscosity).
- 2 Gravimetric sensing: Velocity affected (absolute gas pressure, fluid density, chemical vapors and gases, protein or bacterial attachment in liquid, diffusion through gels, biochemically selective sensing not yet demonstrated).
- 3 Kinetic effects: In gaseous ambient (moving solid blocks, granular powders); in liquid (pumping fluids, trapping and spinning bacteria, mixing (fluids, and for higher collection efficiency at electrochemical electrodes).

Anticipated Technological Developments: Micro-flow systems (sensing and kinetic functions combined on dies or entire wafers for reconstituting dried biochemicals, chemical synthesis, heat redistribution in IC chips); combination of ultrasonic with optical vapor sensing in single structure; use of polymeric propagation structures offering more geometrical freedom; employment in vapor and biomedical sensing systems with disposable elements likely; use of arrays of devices for selectivity and/or multi-measurand determination; low TEMPCO designs; high-resolution position sensing.

Fabrication Details: Present devices employ two unusual technologies -- deposition of low-stress nitride (to 2 micron thickness) and piezoelectric film (to 1 micron thickness). Feature sizes range from 25 microns downward (thinner membranes and smaller transducer periods lead to higher sensitivities). Devices having transverse dimensions from 3 X 3 cm² to 0.15 X 0.05 cm² have been made.

Business Aspects: A particular vapor sensing market of 0.5 million devices identified. A fab house might supply basic devices to others who would apply propriety coatings or special packaging. Patent applications in progress on the technology (U. of California), some of which predates BSAC and so is available for exclusive licensing. Estimated \$0.15-0.20 cost per die with dedicated fab. Relationships with BSAC members possible.

Sketches and Specs of Representative Devices:

Operating frequencies of flexural mode: 0.6 - 10 MHz

Gravimetric sensitivity factor: at least 1000 cm²/g at 2.6 MHz

Short-term noise: approx. 10⁻⁷ ppm

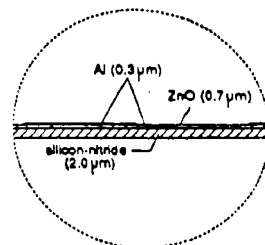
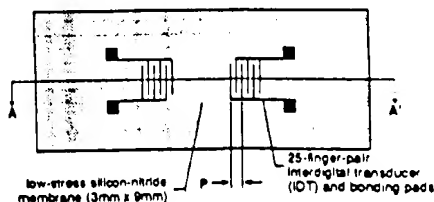
Estimated minimum detectible added mass/area change: 100 pg/cm² water loaded

Differential pressure sensitivity: 0.65 Hz/microbar

Absolute pressure sensitivity: 1.5 kHz/atmosphere (helium)

Viscosity measurement range: from <1 cp to more than 10,000 cp.

Temperature coefficient of delay (uncompensated): -120 ppm/°C



LAMB-WAVE INTERACTIONS WITH THE CHEMICAL, BIOLOGICAL AND PHYSICAL ENVIRONMENT

R. M. WHITE, S. W. WENZEL, B. A. MARTIN, R. M. MORONEY, B. J. COSTELLO, M. A. STRAUB and R. T. HOWE

Berkeley Sensor & Actuator Center,
University of California, Berkeley, CA

Microfabrication processes permit one to form self-supporting micron-thick membranes, in a silicon frame, that support propagating ultrasonic Lamb waves. With membrane thicknesses much smaller than the ultrasonic wavelength, low wave velocities (60-500 m/s) and low operating frequencies (0.6-5 MHz) result. These slow ultrasonic delay lines can be in contact with or immersed in ideal liquids without suffering excessive loss due to radiation. They are also very sensitive to mass loading and can have large wave amplitudes (1000 Å with 10-volt drive).

Fig. 1 shows views of a typical device. Composite membrane thicknesses from one to four microns have been utilized, with wavelengths from 40 to 100 micrometers. Tests and/or analyses of four types of functions have been made:

- Sensing physical quantities such as differential pressure and viscosity.
- Non-selective gravimetric sensing of chemical vapors, and proteins or bacteria in a liquid that contacts the sensor.
- Selective ligand-based gravimetric sensing with antigen-antibody binding or DNA probes.
- Kinetic effects such as moving solid particles or bacteria, and mixing of fluids.

Fig. 2 shows responses to water-glycerol solutions having various viscosities. The linear dependence of insertion loss (corrected for density) on viscosity agrees with theoretical predictions. 7 thicker membranes respond most strongly to viscous dissipation.

Non-selective gravimetric sensing (Fig. 3) has been observed with polymer-coated devices. Analysis shows that the fractional frequency change, which equals the fractional velocity change, is larger for the Lamb-wave gravimetric sensor than for corresponding SAW or bulk-mode sensors. Table lists calculated operating characteristics of a typical device. Note that the minimum calculable detectable mass change per unit area while in contact with water is only 96 pg/cm^2 . We have

successfully detected the non-specific adsorption of protein molecules and bacteria from saline in contact with the device. Tests of selective ligand-based sorption are underway.

Large particle motions result because of the confinement of the wave energy in such a thin region. We have moved nanogram-mass polysilic blocks with RF drive voltages as low as 1.5 V, and have measured block velocities up to 2 cm/s caused by a 10-volt drive. The waves have produced fluid circulation and gently transported biological objects. Mixing experiments in an electrochemical cell are in progress. Because of their relatively large amplitudes, it is also possible to observe these waves visually.

We will describe experimental observations and calculated limiting sensitivities in this paper, together with some possible applications of the Lamb-wave devices.

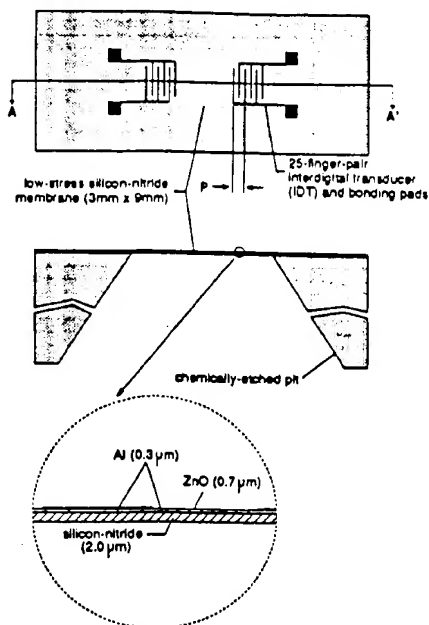


Fig. 1. Typical Lamb-wave delay line. With a 100-micrometer wavelength, equal to the period of the interdigital transducers, p , the device shown has a wave velocity in vacuum of 474 m/s and operates at 4.74 MHz.

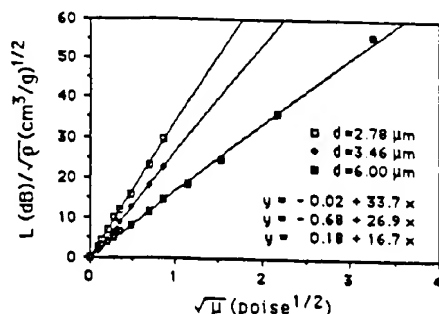


Fig. 2. Insertion loss, L , corrected for density variations, vs. square root of viscosity for devices having three different thicknesses, d .

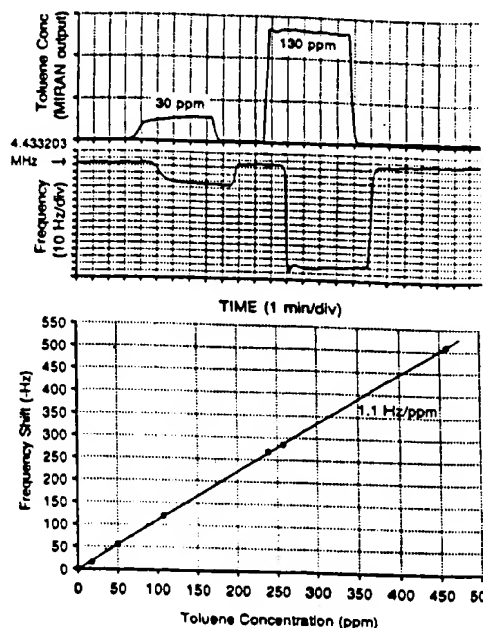


Fig. 3. Toluene vapor sensing with Lamb-wave sensor coated with poly(dimethylsiloxane). Top trace: concentration of toluene in air carrier gas as indicated by MIRAN infrared spectrophotometer. Middle trace: frequency of Lamb-wave sensor (0 minute delay is caused by gas transport through heat exchanger coils). Bottom trace: Frequency shift vs. toluene concentration. Minimum detectable concentration at 3:1 signal-to-noise ratio is 1 ppm; with ethyl cellulose coating it is only 70 ppb.

DEVICE PARAMETERS	
Thicknesses:	
*Silicon nitride (μm)	0.1
Zinc oxide (μm)	0.1
Ground plane (μm)	0.1
Total (μm)	0.3
Transducer:	
*Period (μm)	8
*Mode number	1
Loading conditions:	
*No. loaded surfaces	1
*Fluid sp. gravity	1
*Fluid sp. viscosity	0

OPERATING CHARACTERISTICS	
*Instability (ppm)	
Instability (Hz)	7.72
Wave velocity (m/s):	
Loaded	309
Frequency (MHz):	
Loaded	38
Mass sensitivity (cm ² /g):	
Loaded	-43
Unloaded	-20
Min. detectable loads (pg/cm ²):	
Mass/area (unloaded)	45
Mass/area (loaded)	96

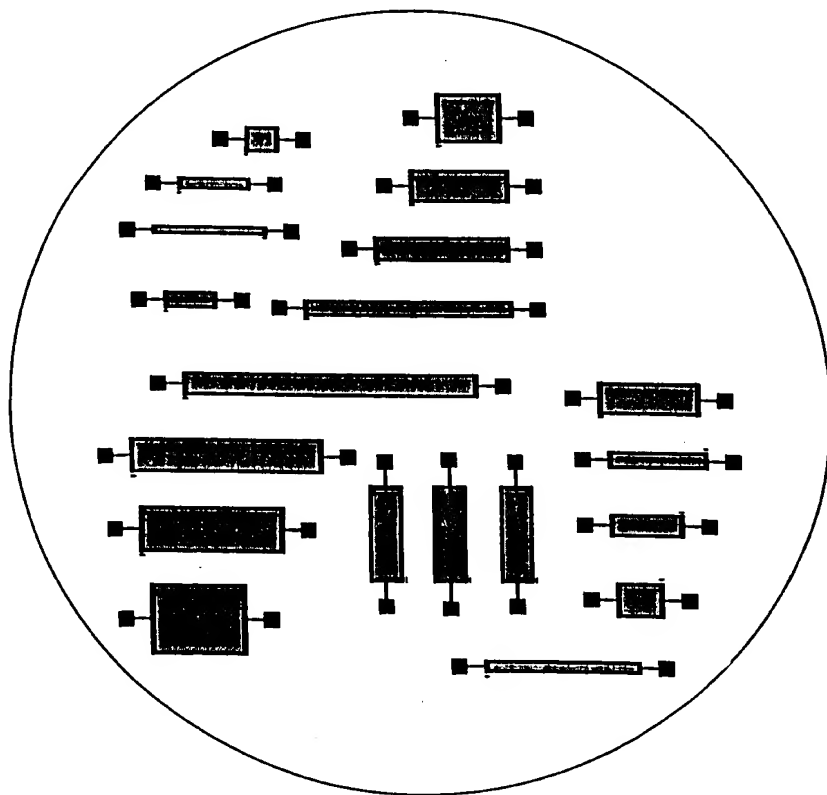
Table 1. Calculated sensitivities of optimized Lamb-wave sensor. Asterisk denotes quantity to set. Dimensions are in micrometers. One surface of device is assumed to be in contact with non-viscous fluid having density of water ("loaded") in a vacuum ("unloaded"). Mass sensitivity is ratio of fractional frequency or velocity shift to added mass per unit area.

BSAC Lamb Wave References

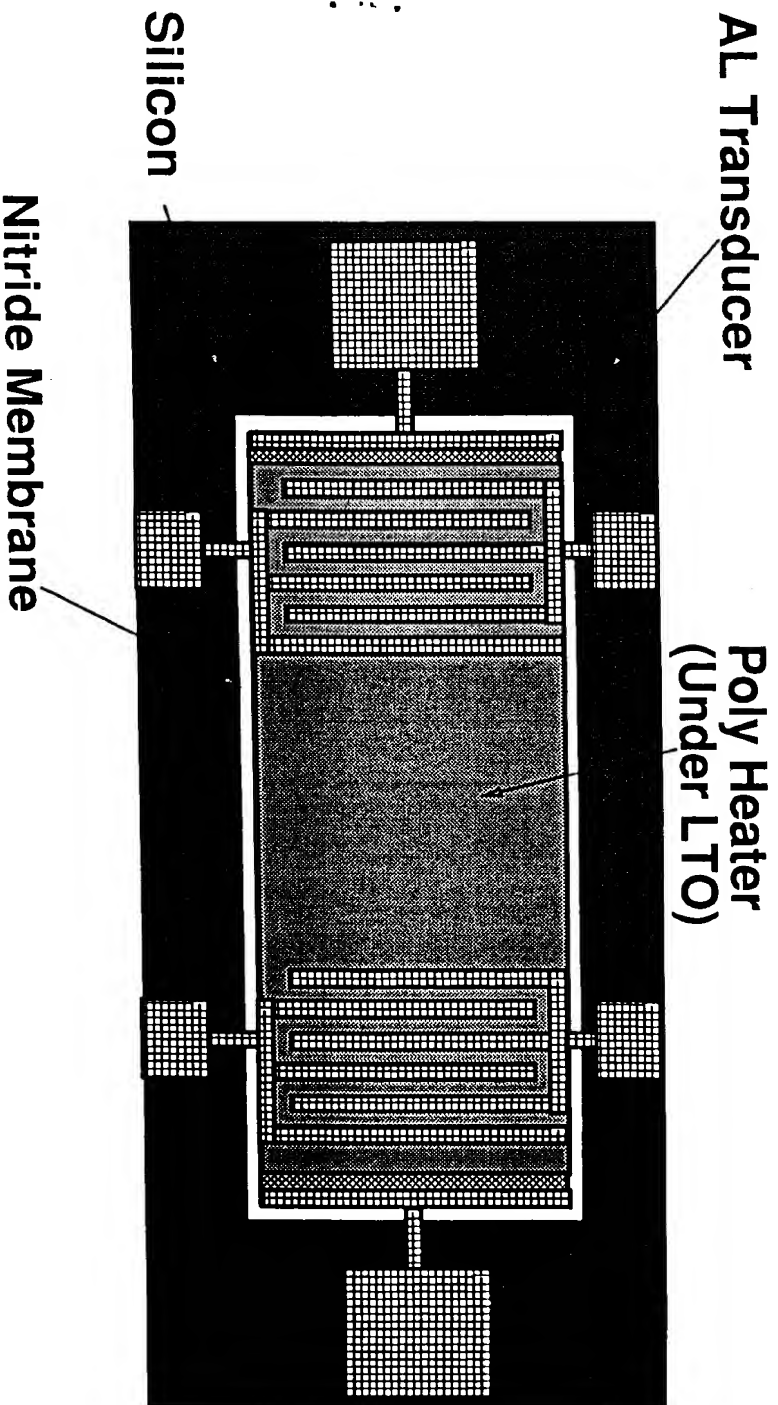
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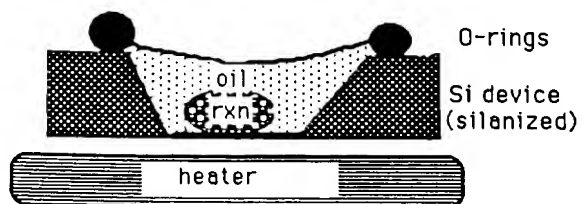
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POLYSILICON HEATING ELEMENT TEST STRUCTURES



BIOCHEMICAL MICROFLOW CHAMBER (TOP VIEW)





Example: device volume = 50 μ l
reaction volume = 30 μ l
oil = 100 μ l

BIOCHEMICAL MICROFLOW CHAMBER

